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Nature beats nurture: a case study of the physiological fitness of free-living and laboratory-reared male *Anopheles gambiae s.l.*

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Summary

Laboratory experimentation forms the basis for most of our knowledge of the biology of many organisms, in particular insects. However, the accuracy with which laboratory-derived estimates of insect life history and behaviour can predict their fitness and population dynamics in the wild is rarely validated. Such comparison is especially important in cases where laboratory-derived information is used to formulate and implement strategies for the genetic control of insects in nature. We have conducted a comparative study of the reproductive potential and life history of male *Anopheles gambiae* Gilies *sensu lato* mosquitoes from both standardized laboratory conditions and from natural field settings. We measured three indirect indicators of male mosquito fitness: energetic reserves, body size and survival, in a bid to determine whether the demographics and energetic limitations of wild males can be correctly predicted from their laboratory counterparts. Crucially, the body size and lipid reserves of wild males were substantially greater than those reared under standard laboratory conditions. We caution that the energetic limitations of insects as identified in the laboratory may underestimate their resilience in the wild, and discuss the implications of this phenomenon with respect to vector-borne disease control programmes based on genetic control of mosquitoes.

Key words: male *Anopheles*, insect fitness, laboratory colonization, genetically modified mosquito, energetic reserves.

Introduction

An inherent feature of laboratory-based experimentation is the quest to limit extraneous sources of variation that may obscure detection of relationships between an outcome variable and hypothetical causative or correlated factors of interest. This usually requires studying organisms in simplified environments; potentially creating bias generally deemed acceptable when weighed against the powerful hypothesis testing these simplifications permit. However, when the aim of laboratory experiments is to estimate parameters to guide the implementation of interventions aimed at natural populations, and whether to protect or suppress them, it is essential to know how closely the physiology and behaviour of laboratory-maintained individuals represent those from the wild. The task of contrasting the responses of laboratory-reared and free-living organisms has held a low profile, but must now be reprioritized to assist the numerous animal and plant population control programmes that rely on releases of captive-reared individuals.

Much of our knowledge of insect ecology and evolution comes from laboratory experimentation. However, the accuracy with which these laboratory-derived estimates of insect life history and behaviour can predict the fitness and population dynamics of insects in the wild is uncertain. Unlike homeotherms, the development and demography of insects is heavily regulated by climate and other environmental variables (Carey, 2001), and can also vary substantially in response to subtle differences in diet (Chang, 2004; Gary and Foster, 2001; Held and Potter, 2004; Jorgensen and Toft, 1997; Straif and Beier, 1996). Given their dependence on environmental variation, behavioural and life-history traits documented under standardized laboratory conditions could grossly misrepresent the complexity and norms of insect behaviours. Critically, laboratory studies using insects reared in captivity may not represent the resilience of their populations to natural disturbances and/or human interventions.

In the case of insect vectors of disease, inappropriate extrapolation of laboratory results could have substantial economic and public health implications. The recent development of genetically modified (GM) *Anopheles* mosquitoes that block the development of malaria parasites

(Christophides, 2005; de Lara Capurro et al., 2000; Ito et al., 2002; Tabachnick, 2003), and the use of sterile insects to suppress pest population growth (Benedict and Robinson, 2003; Dyck et al., 2005), serve as excellent examples of this issue. Both these approaches require the release of laboratory-reared individuals in the wild, with the GM approach seeking to reduce malaria by introducing a parasite refractory gene into natural populations, and the Sterile Insect approach to suppress population growth by inducing wild females to mate with infertile males. Ethically, only male mosquitoes could be released in such programmes as the release of more blood-feeding females would at best increase the biting nuisance, or at worst, the transmission of other vector-borne pathogens and possibly even malaria itself if transgenic females are not 100% refractory.

The mating ability and survival of laboratory-reared, GM or sterile males when released into the wild is thus critical to the success of these enterprises. However, comparisons of the fitness of genetically modified and wild-type mosquitoes have thus far been made only under laboratory conditions (Catteruccia et al., 2003; Irvin et al., 2004; Moreira et al., 2000; Moreira et al., 2004). Colonization can alter the mating behaviour of laboratory-reared mosquitoes and generate selection for assortative mating traits. The evolution of assortative mating preferences reduces ability to mate with wild-type female conspecifics, and can occur in as few as three generations of laboratory maintenance (Reisen, 2003). Direct field tests of the competitiveness of laboratory-reared genetically modified mosquitoes when pitted against wild males must necessarily wait until concerns regarding the ethics, biosecurity and efficacy of this approach are resolved (Knols et al., 2007; Mshinda et al., 2004). In the meantime, substantial progress towards assessing the effectiveness of the GM and Sterile Insect approach could be made by contrasting the fitness of male mosquitoes when mass-reared in the laboratory, and allowed to forage freely in nature; this to our knowledge has never been conducted on male African *Anopheles*.

The reproductive potential and fitness of male mosquitoes can be indirectly measured by their energetic reserves as adults (Briegel, 1990; Van Handel, 1984). These reserves, accumulated during larval development and/or from blood or sugar-feeding as adults (Briegel, 2003; Foster, 1995), are critical determinants of adult survival and mating ability (Briegel, 1990; Timmermann and Briegel, 1999; Van Handel, 1988). Three key energetic reserves of adult mosquitoes are lipids, glycogen and sugar. Lipids are required for long-term maintenance (e.g. survival), and are primarily acquired from feeding during larval development, and sugar feeding as adults (Briegel et al., 2001; Van Handel, 1984). Flight is a requirement for mosquito mating, an activity fuelled by sugars or glycogens, derived from sucrose or its components fructose and glucose, in nectar, honeydew and fruit juices (Briegel, 2003; Foster, 1995; Nayar and Sauerman, 1977; Rowley and Graham, 1968; Van Handel, 1984). Body size is another indirect measure of mosquito reproductive success, with several studies showing that larger individuals have greater reproductive success (Ng'habi et al., 2005; Takken et al., 1998; Yuval et al., 1993).

Given previous observations of poor survival and reproduction in laboratory-reared mosquitoes when released into the wild (Ferguson et al., 2005), it is often assumed that free-living insects are subject to much harsher environmental conditions and may generally be smaller in size and have lower levels of energetic reserves than those reared in standardized controlled environments. This suggests that mosquitoes reared in standardized laboratory conditions should be better provisioned to out-compete wild individuals upon release. If this is not true, any fitness cost conferred by a refractory gene (Catteruccia et al., 2003; Irvin et al., 2004; Moreira et al., 2004), or irradiation (Helinski et al., 2006b) in the case of the sterile insect technique, will be further inflated by the poorer physiological condition of laboratory-reared mosquitoes. In the present study we investigated how key nutritional resources and body size vary between laboratory-reared and free-living male mosquitoes from southern Tanzania. We focused on male *An. gambiae s.s.* Giles and its sibling species *An. arabiensis* Patton, because little is known about the biology of this sex (Ferguson et al., 2005), and because these species are the most important vectors of malaria in Africa (Gillies and DeMeillon, 1968; White, 1974) and thus a leading target for control measures based on the release of genetically modified (Ito et al., 2002; Riehle et al., 2003) and/or sterile males (Helinski et al., 2006a). Consequently there is an urgent need to understand the life history and performance of free-living male *An. gambiae s.l.*, and evaluate the extent to which their behaviour, physiology and reproductive potential can be inferred from laboratory observation.

Materials and methods

Field collection and dissections

Anopheles gambiae Giles *sensu lato* were collected in Lupiro village (8°23′39,49 South, 36°40′21,38 East) in the Kilombero valley of Tanzania (Charlwood et al., 1995). Over a 4-week period in 2005 (mid May–mid June), we conducted daily resting catches in the morning (06:00–08:00 h) in approximately 10 houses and outdoor toilets to collect *Anopheles* mosquitoes. Daily temperatures during this collection period fluctuated between 28–30°C, which matched the ambient conditions under which our laboratory colony was maintained (field site was located at the same altitude, approximately a 1-h drive from our laboratory). Males visually identified as belonging to the *An. gambiae s.l.* species complex (Gillies and DeMeillon, 1968) were kept for dissection, which was done within 1 h after collection. Mosquitoes were killed by shaking them in a holding cup; one leg was then removed from each male *An. gambiae s.l.* and stored in an Eppendorf tube containing silica gel for genotypic identification to sibling species level using PCR (Scott et al., 1993).

In addition, one wing was removed and measured under a dissecting microscope fitted with an ocular micrometer (1 unit=0.35 mm). Mosquito wing length is often used as a proxy for body size as it is a fixed trait that is relatively easy to measure, and is positively correlated with body mass in most species (Koella and Lyimo, 1996; Nasci, 1990; Siegel et al., 1992). The relationship between wing length and body mass is variable, and its exact nature can differ between mosquitoes of different species, strain and rearing background (Nasci, 1990; Siegel et al., 1992; Siegel et al., 1994). Despite this limitation, Anopheline mosquito wing length has consistently been shown

to be a significant predictor of traits such as fecundity and survival (Ameneshewa and Service, 1996; Hogg et al., 1996; Kittayapong et al., 1992; Lehmann et al., 2006; Lyimo and Takken, 1993), and thus was selected as a useful approximator of mosquito fitness for our purposes.

After wing removal, the remainder of the mosquito body was placed in a drop of phosphate-buffered saline (PBS) on a cavity microscope slide. The reproductive system of males was removed using dissecting pins under a dissecting microscope (10×), and examined under a compound microscope (50×). Three key features of the male reproductive system that have been associated with male *An. gambiae* age (Huho et al., 2006) were observed and scored: the number of spermatocysts in the testes, proportion of the testes occupied by the sperm reservoir, and presence or absence of a clear border surrounding the edge of the accessory gland. Remaining male body parts and fluids were washed into a test tube using 100 μl 100% ethanol. In the field, these tubes were heated at approximately 90°C for 10 min over a heating block in order to temporarily fix and preserve energetic reserves for subsequent biochemical analysis in the laboratory. Following this protocol, samples can be stored for up to 2 weeks at room temperature before being processed (H. Briegel, personal communication).

Mosquito species identification

DNA was extracted from legs of individual wild-caught male *An. gambiae* by placing them individually in an Eppendorf tube containing 15 μl of Tris-EDTA (TE) buffer, and then crushing them using a micropestle. 3 μl of this solution was used for DNA extraction. A master mix containing DNA templates for the *An. gambiae* species complex was prepared, and added to each DNA sample to initiate the PCR (Scott et al., 1993). Only two *An. gambiae s.l.* species were represented within our field sample, namely *An. arabiensis* and *An. gambiae s.s.* Giles.

Laboratory reared mosquitoes

Male *An. gambiae s.s.*, from the insectary at Ifakara Health Research Development Centre, were used for comparison with wild mosquitoes. These mosquitoes originated from a sample colonized from wild individuals collected in 1996 at Njage village (8°20′00,05 South, 36°05′30,57 East). Since then, these mosquitoes have been reared in laboratory conditions perceived as ideal for survival and reproduction. As larvae, they are maintained on a standard diet of TetraMin® fish food (Tetra GmbH, Melle, Germany) at densities of 150–200 larvae per 100 ml of water in a larval tray (32 cm×12 cm×15 cm). Upon emergence, adult males were pooled in a separate cage and maintained on a 10% glucose solution, at ambient conditions (approximately 28–30°C, 70–80% relative humidity) and a photoperiod of 14 h:10 h (L:D). From these cohorts of males, groups of different age (1–20 days) were randomly sampled and subjected to biochemical analysis to assess their energetic reserves. Their body size was also estimated from their wing length as described above. As with the wild-collected mosquitoes, laboratory-reared males had one leg, one wing and their reproductive system removed before their remaining parts were fixed in ethanol and stored for further biochemical analysis. *Anopheles gambiae s.s.* was the only captive reference strain available in the laboratory group.

Laboratory quantification of sugars, glycogen and lipids

We determined the contents of three key energetic reserves in field- and laboratory-collected mosquitoes using a spectrophotometric method originally devised by Van Handel (Van Handel, 1985a; Van Handel, 1985b). Standard curves for converting absorbency readings into quantities of lipids, sugars and glycogen were obtained from two replicate series of experiments, in which the absorbency of known concentrations of each reserve were measured.

Age grading of wild male mosquitoes

Previously we have shown that an age-grading method based on male reproductive morphology originally devised for Asian *Anopheles* (Mahmood and Reisen, 1982; Mahmood and Reisen, 1994) can be successfully adapted for male *An. gambiae s.s.* (Huho et al., 2006). Information on the number of spermatocysts, relative size of the sperm reservoir, and presence of a clear area surrounding male accessory glands was used to classify male *An. gambiae s.s.* into age categories of ‘young’ (⩽4 days post emergence) and old (>4 days) with 89% accuracy (Huho et al., 2006). We applied this model here to age-grade wild-collected males, and test for any association between age and reserve abundance.

Data analysis

Preliminary analysis of the total glycogen, sugar and lipid content of male mosquitoes indicated that these reserves did not follow a normal distribution (Kolmogorov–Smirnov normality test, $P<0.001$). Consequently we used the non-parametric Mann–Whitney test for two independent samples to test for differences in reserve levels between the following treatment groups: (1) laboratory-reared and field-collected males, (2) field-collected *An. gambiae s.s.* and *An. arabiensis* males, and (3) males of different age categories (two age groups: ⩽4 days post-emergence, or older). Laboratory-reared males were excluded from the analysis of between-species variation in reserve levels to avoid confounding species differences with those generated by rearing condition (as only one species, *An. gambiae s.s.* was represented in the laboratory group). Relationships between male body size and reserve levels were investigated using Spearman’s correlation coefficient (non-parametric), and analysis of variance (ANOVA) was used to test if there were differences in body size between treatment groups that could account for observed differences in reserve levels. All data were statistically analyzed using SPSS (version 11.5). Unless otherwise stated, numbers in parentheses following means represent one standard error (s.e.m.).

Results

A total of 482 male *An. gambiae s.l.* were captured over 28 days of resting catches. Of these, 459 were successfully identified to species level by PCR. *Anopheles gambiae s.s.* was the dominant species in this sample (86.7% *An. gambiae s.s. vs* 13.3% *An. arabiensis*). A sample of 190 *An. gambiae s.s.* males was obtained from our laboratory colony and analyzed for comparison with this field sample.

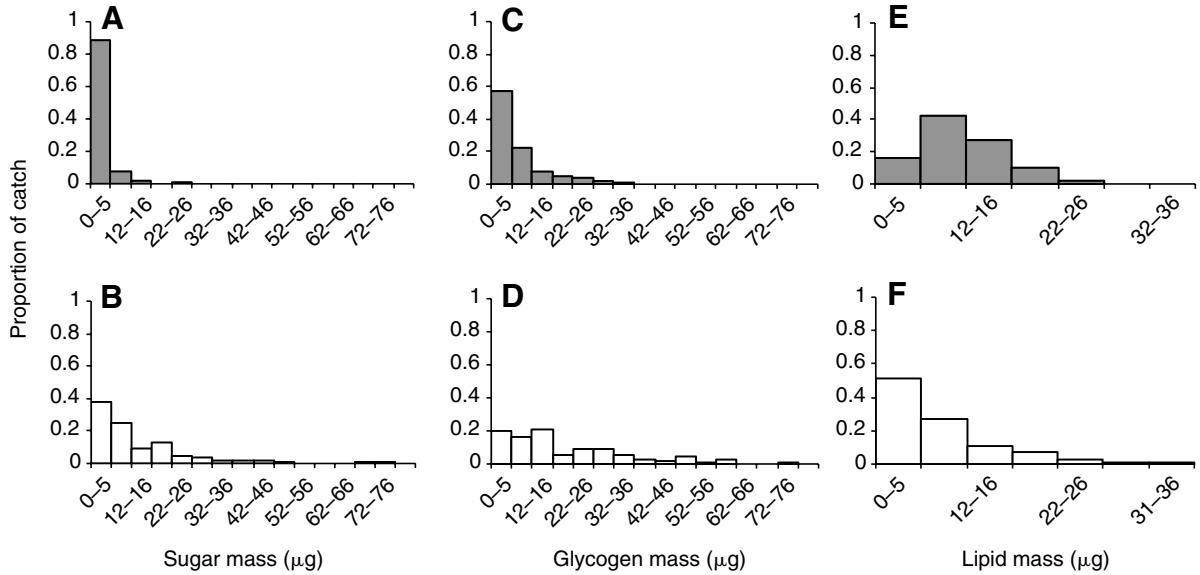

Fig. 1. Frequency distribution of masses of three key energy reserves detected in field-collected (A,C,E; grey bars) and laboratory-reared (B,D,F; white bars) male *An. gambiae s.s.*

Energetic reserves in laboratory reared and wild male An. gambiae s.s.

The majority of males collected in the field had no detectable sugars (70%) and many also tested negative for glycogen (30%). In contrast, 83% and 90% of laboratory-reared males tested positive for sugars and glycogen, respectively. Males from the laboratory were 12 times more likely to test positive for sugar than the field group [χ^2=158.15, P<0.01, odds ratio=12.93, 95% CI: (8.31–20.13)], and four times more likely to test positive for glycogen [χ^2=34.29, P<0.01, odds ratio=4.32, 95% CI: (2.57–7.24)]. In contrast, lipids were detected at a much higher frequency in wild than in laboratory-reared males [97.3% *vs* 78% prevalent in wild and laboratory males, respectively: χ^2=54.78, P<0.01, odds ratio=9.98, 95% CI: (5.0–19.91)].

Not only the prevalence, but also the abundance, of sugars was higher in laboratory-reared male *An. gambiae s.s.* than in their wild conspecifics (Mann–Whitney U=13397.0, P<0.01, $\text{Median}_{\text{LAB}}$=8.01 μg, $\text{Median}_{\text{FIELD}}$=0 μg, Fig. 1A,B). Similarly, glycogen content was higher in laboratory-reared males, being on average three times greater than the amount found in wild males (Mann–Whitney U=19783.5, P<0.01, $\text{Median}_{\text{LAB}}$= 15.26 μg, $\text{Median}_{\text{FIELD}}$=4.21 μg, Fig. 1C,D). In contrast, lipid content in wild *An. gambiae s.s*. males was more than twice that of laboratory-reared individuals (Mann–Whitney U=23035, P<0.01, $\text{Median}_{\text{LAB}}$=4. 6 μg, $\text{Median}_{\text{FIELD}}$=9.6 μg, Fig. 1E,F).

Adult body size also varied significantly between laboratory-reared and field-collected *An. gambiae s.s.* ($F_{1,554}$=436.77, P<0.001). Wild males were approximately 17% larger than laboratory-reared individuals [Mean_{LAB}=2.17 mm (0.011), $\text{Mean}_{\text{FIELD}}$=2.54 mm (0.010)]. Body size was substantially more variable in field-collected males (range 1.86–3.14 mm) than in the laboratory-reared males (range 1.89–2.57 mm). Male body size was positively correlated with lipid stores in both laboratory-reared and wild male *An. gambiae s.s.* (Table 1; Fig. 2C). In contrast, the amount of glycogen and sugars in males was not associated with the body size of either laboratory or field-collected males (Table 1; Fig. 2A,B).

Between-species differences in energetic reserves of wild collected mosquitoes

Restricting analysis to field-collected mosquitoes, the quantity of stored reserves did not vary between *An. arabiensis* and *An. gambiae s.s.* (Table 2). Despite the lack of variation in reserve abundance between mosquito species, *An. arabiensis* males were significantly larger than *An. gambiae s.s.* [$F_{1,457}$=11.38, P<0.01, $\text{Mean}_{\text{ARABIENSIS}}$=2.63 mm (0.024), $\text{Mean}_{\text{GAMBIAE}}$=2.54 mm (0.010)]. Thus for a given unit of body length, *An. gambiae s.s.* contained a higher abundance of

Table 1. *Correlations between male mosquito body size and energetic reserves*

		Spearman's correlation with body size		
Background	Species	Sugars	Lipids	Glycogen
Laboratory	*An. gambiae s.s.*	–0.03	0.18*	–0.13
Field	*An. gambiae s.s.*	0.04	0.12*	–0.02
	An. arabiensis	0.289*	0.14	–0.24

*Statistically significant correlations (P<0.05).

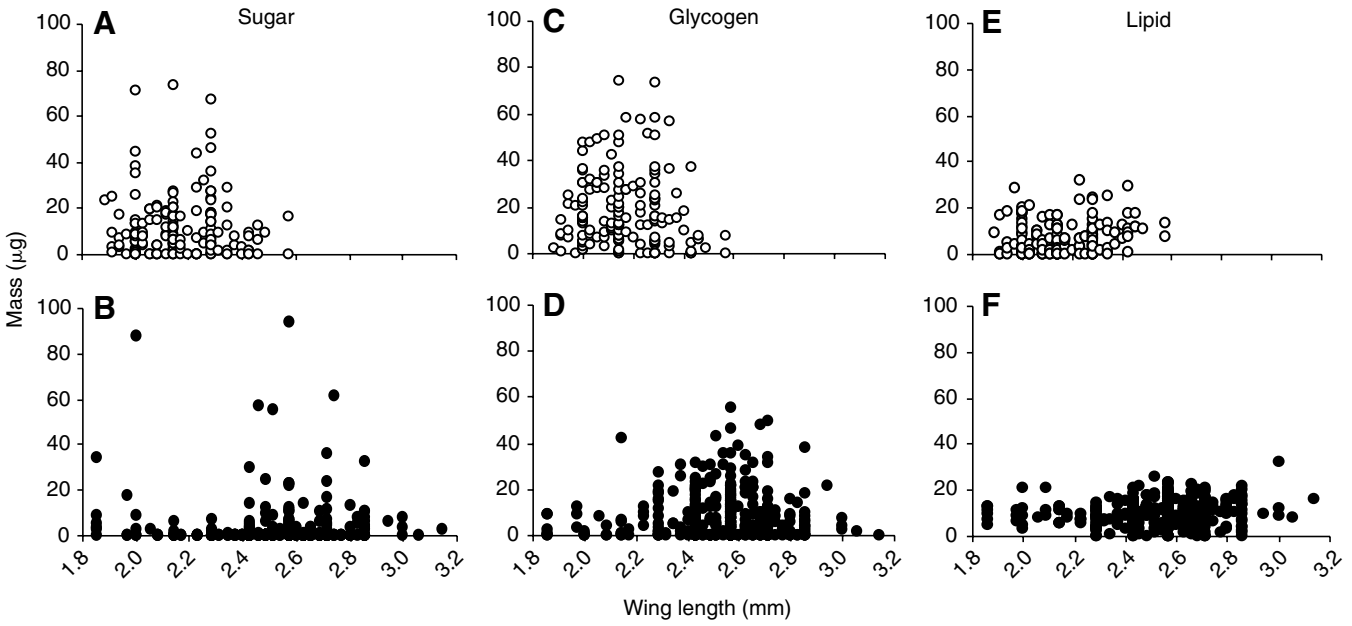

Fig. 2. Relationship between body size and three key energetic reserves in laboratory-reared (A,C,E; open circles) and free-living (B,D,F; black circles) male *An. gambiae s.s.*

energetic reserves than *An. arabiensis*. Body size was positively correlated with sugar abundance in *An. arabiensis* but not in *An. gambiae*, as detailed above (Table 1). Lipids were positively correlated with body size in field-collected *An. gambiae* but not *An. arabiensis* (Fig. 3C). Neither species showed any association between body size and glycogen (Table 1; Fig. 3B).

Variation in energetic reserves with age

The age of laboratory-reared males was known with certainty because this was tracked from emergence. To facilitate comparison with age grades available for the field sample, we pooled our laboratory sample into two age groups of 'young' (≤4 days post-emergence) and 'old' (>4 days post-emergence). The morphologically based method we applied to age-grade our sample of wild males into similar categories is approximately 89% accurate (Huho et al., 2006). Our aim was to test whether energetic provisions change with age in both wild and laboratory-reared males. One potentially confounding factor when testing for age-related changes, or lack thereof, is size-selective mortality. If small males die earlier than large males, the older age group, both in the laboratory and the field, may be disproportionately represented by large males who inherently have greater reserve levels; this phenomenon could obscure any decline in reserve abundance with age. To rule this out, we first tested whether the body size of young and old males varied. We found that the average body size of 'old' males was indeed greater than that of 'young' males in both field ($F_{1,389}$=12.11, P<0.01) and laboratory samples ($F_{1,157}$=4.78, P=0.03), indicating size-selective mortality is operating in both populations. We then sub-selected from within our field-collected *An. gambiae s.s.*, *An. arabiensis*, and lab-collected *An. gambiae s.s.*, to obtain samples of 'young' and 'old' males of approximately equal body size. This was done by calculating the mean body size for males in each of the three groups, and eliminating individuals whose body size fell outside one standard deviation of this mean. Subsequent statistical analysis revealed no statistical difference in body size between 'young' and 'old' males within these sub-samples (P=0.43 for field *An. arabiensis*; P=0.79 for field *An. gambiae s.s.*; P=0.77 for laboratory *An. gambiae s.s.*). Within these size-restricted groups, there was no difference in sugar or lipid mass between 'young' and 'old' males (Table 3). However, there was a substantial increase in glycogen content in older males within field-collected the *An. gambiae s.s.* sample (Table 3); this observation was not evident within the laboratory group or wild *An. arabiensis* males.

Discussion

In this study we show that male *Anopheles* mosquitoes allowed to forage freely in nature outperform individuals reared in laboratory conditions with respect to at least two key determinants of adult survival: body size and lipid reserves. In contrast, and perhaps unsurprisingly given their *ad libitum* glucose diet, laboratory-reared males had substantially greater reserves of sugar and glycogen than wild males. These findings challenge the notion that measures of insect fitness and reproductive potential will be upwardly biased in laboratory studies, and stimulate re-evaluation of the optimal rearing conditions for mosquito development and maintenance. Interestingly, these conclusions differ from those reported for female mosquitoes, in which levels of sugar, glycogen and lipid were always higher in laboratory-reared than field collected individuals (Day and Van Handel, 1986; Klowden, 1988). This discrepancy between studies of males and females suggests the existence of sex-specific variation in mosquito energetic budget, and that males may require a broader range of nutritional resources to maximize their energetic reserves than females.

While the higher sugar and glycogen content of laboratory-reared mosquitoes was expected, the greater lipid reserves of wild male *Anopheles* was not. Unlike wild male mosquitoes, *An.*

Table 2. *Median value of energetic reserves in males of two Anopheline species with different rearing backgrounds*

	Median value of reserve (μg)		
	An. gambiae s.s.		*An. arabiensis*
	Laboratory	Field	Field
Sugars	8.01	0	0
Glycogen	15.26	4.21	2.62
Lipids	4.54	9.67	10.36

In all cases, reserve levels in field-collected *An. gambiae s.s.* were statistically different from those reared in the laboratory ($P<0.05$). Reserve levels were not statistically different between field-collected *An. gambiae s.s*. and *An. arabiensis* (described in text).

gambiae s.s. maintained in the laboratory had a guaranteed supply of sugar at all times of day, and were rendered largely inactive due the limited confines of their cages. The higher accumulation of sugars and glycogen under these conditions in contrast to free-living mosquitoes is thus not surprising, and suggests that wild males do not sugar-feed to repletion, probably due to limitations in the availability of sugar sources. Alternatively, free-living mosquitoes may sugar-feed less during the night than laboratory males, resulting in a lower detectable of carbohydrate reserves when they were sampled in the early morning hours.

We hypothesize that the larger lipid stores of field-collected males is a by-product of their larger body size. Female anophelines are known to accumulate lipids in a size-dependent manner (Briegel, 1990); this observation is supported here for males. As mosquito body size is determined almost entirely by larval nutrition and microclimate (Briegel, 1990; Timmermann and Briegel, 1999), the artificial larval habitats we created in the laboratory may have been of lower quality to those of males sampled in the wild. However, it is not yet possible to conclude whether our results indicate that natural conditions are generally more or less 'harsh' than the laboratory. The greater body size and lipid stores of field mosquitoes could imply that natural larval habitats are generally of higher quality than those in the laboratory. Alternatively it could be that rates of larval mortality in the field are substantially higher than in the laboratory, such that the small percentage of individuals that do survive can rapidly accrue resources without interference from competitors (Agudelo-Silva and Spielman, 1992). 'Common garden' experiments in which mosquitoes from the field are reared under laboratory conditions, or *vice versa*, will help this resolve this issue. Alternatively, these results may have little to do with the relative benignity of either setting, but reflect that long-term evolutionary adaptation of *Anopheles* to field rather than laboratory conditions. Regardless of the particular ecological or evolutionary mechanism, our results suggest that mosquitoes reared under standard laboratory conditions are not of equal quality to free-living male mosquitoes with respect to at least two key determinants of lifetime reproductive fitness.

In light of these findings, what can we conclude about the likely success of laboratory-reared *versus* wild male mosquitoes when competing against each other in nature? Ultimately the relative success of male mosquitoes is determined by their lifetime mating success; this is a composite measure, depending on both their ability to obtain mates on a particular swarming event, and the number of swarming events in which they can participate (correlated with survival). With respect to the first component of male reproductive success, sugars and glycogen are known to determine male mating success in a swarm, with the ability to initiate and sustain swarming being positively associated with carbohydrates reserves (Briegel et al., 2001; Nayar and Sauerman, 1977; Rowley and Graham, 1968; Yuval et al., 1994). Thus the higher abundance of sugars and glycogen

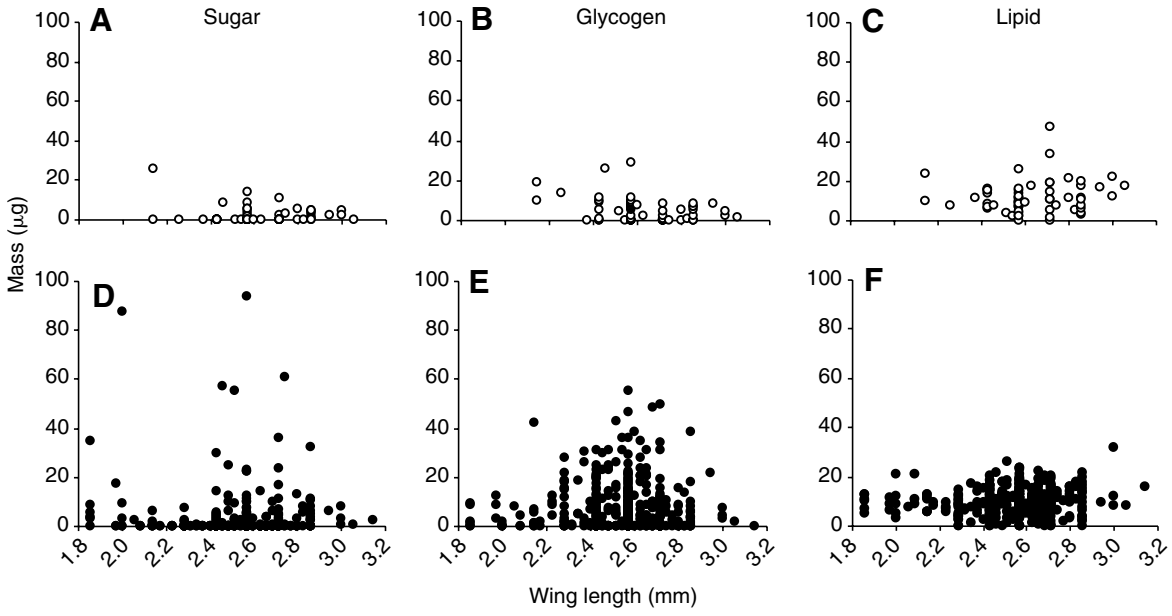

Fig. 3. Relationship between body size and three key energetic reserves in free-living male *An. arabiensis* (A–C; open circles) and *An. gambiae s.s.* (D–F; black circles).

Table 3. *Median values of reserves in three sub-samples of male mosquitoes, selected to generate groups in which the body size of 'young' and 'old' individuals were similar*

Group	Age	Median reserves (μg)			
		N^R	Sugars	Glycogen	Lipids
An. gambiae s.s. (L)	Young	51	8.37	15.72	4.95
	Old	49	9.70	15.32	4.36
An. gambiae s.s. (F)	Young	110	0	3.77*	9.34
	Old	196	0	5.59*	9.92
An. arabiensis (F)	Young	10	0	3.74	7.66
	Old	27	0	2.22	10.07

N^R, number of males included in these size-restricted samples; F, males that were collected in the field; L, males obtained from a laboratory colony.

*Statistically significant difference between the abundance of a reserve in young and old males (Mann–Whitney *U* test, $P<0.05$).

in laboratory-reared males may predispose them towards greater competitive success in a swarm. However, males from the field have substantially greater body size than those from the laboratory, and this trait that has also been associated with greater competitive success in a swarm in some (Ng'habi et al., 2005; Yuval et al., 1994) but not all studies (Charlwood et al., 2002).

In terms of the second component of male mosquito lifetime reproductive fitness, adult survival, free-living males should have an advantage because they have greater lipid stores than laboratory-derived males. Several studies have shown that long-term survival is positively associated with lipid abundance in mosquitoes (Briegel, 1990; Service, 1987; Van Handel, 1984) and in other insects such as *Drosophila melanogaster* Meigen (Service, 1987). Adult body size is also positively associated with survival, with the present study and others showing that larger mosquitoes live longer (Ameneshewa and Service, 1996; Hawley, 1985; Reisen et al., 1984). Thus both the body size and lipid provisioning of wild males incline them towards substantially greater survival than laboratory-reared individuals. If this advantage outweighs the possibly shorter-term benefit of relatively higher sugar content, it is likely that free-living males will have higher physiologically determined reproductive potential than their laboratory-reared counterparts.

It is generally assumed that in nature, male mosquitoes depend upon sugars from plant juices for longevity and other reproductive functions (Foster, 1995; Van Handel, 1984; Van Handel and Day, 1990; Yuval et al., 1994). In this study, however, we found only small amounts of free sugars in field-collected males, with the vast majority having no detectable levels of sugar. A finding similar to these observations was obtained for five species of mosquitoes from Florida analyzed by gas chromatography (Burkett et al., 1999; Burkett et al., 1998). This contrasts with studies of *Anopheles freeborni* Aitken, which found substantial levels of sugars in males sampled in resting catches (Yuval et al., 1994). *Anopheles gambiae* males may have a lower dependence on sugar feeding or may replenish their reserves at different times of day than *An. freeborni*. Consequently, the importance of sugar-feeding for male *Anopheles* remains an open question and likely varies substantially between species, populations, and habitats (Foster, 1995). Further comparative analyses of the physiology of *Anopheles* species in different environments with different floral sugar resources will help resolve this issue.

We caution that we may have underestimated the proportion of wild males feeding on sugar in this study, as the anthrone technique used here may not reliably detect very low levels of sugar. An alternative method is gas chromatography, which has also been successfully used to measure sugar composition and quantity in mosquito crops (Burkett et al., 1999; Burkett et al., 1998), and may be able to detect sugars at lower quantities. However, unlike the anthrone technique, gas chromatography does not easily facilitate simultaneous measurement of additional nutritional reserves (e.g. lipids, glycogen and protein), and requires analysis equipment that is substantially more expensive and not yet typically available within field settings in Africa. While use of gas chromatography may have increased the proportion of wild males that we considered to be positive for sugars, it would not have qualitatively changed our main conclusion: sugars were much more abundant in laboratory than wild-caught males. Future studies could make use of this more specific gas chromatographic method in order to identify the source of sugars consumed by males (e.g. nectar, plant juices, honeydew), and their relative abundance in our field site.

There were no measurable between-species differences in the abundance of energetic reserves in wild *An. gambiae s.s.* and *An. arabiensis*. Interestingly, reserve levels were constant across these two species, despite the fact that *An. arabiensis* was significantly larger than *An. gambiae s.s*. As lipid levels, both in this study and others, are known to increase with body size (Yuval et al., 1994), it is unclear why *An. arabiensis* did not gain an energetic advantage from its increased body size. One possibility stems from the observation that *An. arabiensis* generally store more water than *An. gambiae* (M. Kirby, personal communication); this feature may explain why they are capable of tolerating drier conditions than *An. gambiae s.s.* (Coluzzi et al., 1979). Thus *An. arabiensis* may devote a smaller proportion of its total body volume to the storage of energetic reserves than does *An. gambiae*, in order to increase its capacity for water storage.

Energetic reserves changed little with male mosquito age; the only observed difference was an age-related increase in glycogen in field-collected *An. gambiae s.s.* It is unclear why this species' laboratory-reared counterparts did not exhibit a

similar increase in this resource with age. One possibility is that sugar resources were so readily abundant to laboratory males that this resource became saturated in their tissues early in life, and simply could not increase further as they aged. Further analyses of male mosquito resource use and energy budget in nature will help identify additional proximate physiological markers of their survival and reproductive success. We note that glycogen is used primarily to fuel mosquito flight (Briegel et al., 2001). The fact that this resource increased with age in the *An. gambiae s.s.* field groups suggests that older males should be equally or even more capable of swarming and dispersal than young males, and thus male reproductive fitness may not decrease with age.

Our findings highlight the importance of validating laboratory-derived estimates of insect physiology and fitness within a field-realistic context. We have shown that indirect estimates of male mosquito fitness as obtained from measurement of body size and energetic reserves vary between field and laboratory populations, and not in a consistent direction (e.g. laboratory mosquitoes do not always have higher or lower reserve levels than field mosquitoes). Specifically, our findings suggest that if one is to release laboratory-reared male mosquitoes of this stature (small and with lower lipid reserves) the likelihood of surviving as long as their wild counterparts may be reduced unless they can build up lipid reserves rapidly. We caution that although the laboratory conditions employed in this study are generally typical of *An. gambiae* laboratory rearing conditions, they do not necessarily represent every permutation of them. Differences in temperature, larval density and food provisioning in the laboratory have been shown to impact adult *Anopheles* size and survival (Lyimo and Takken, 1993; Ng'habi et al., 2005; Reisen and Emory, 1977). We did not systematically evaluate the performance of mosquitoes reared under different laboratory regimes to those in the wild, but rather those reared under one set of conditions that we assume to be broadly representative of how *An. gambiae* are reared in laboratory colonies throughout the world. One slight discrepancy is that we chose to maintain mosquitoes from our laboratory colony at ambient temperatures (28–30°C) which, although well within the acceptable range of *An. gambiae*, is slightly higher than the 27±1°C frequently reported in some laboratory colonies. Our laboratory colony was deliberately maintained under the same ambient conditions as our field populations, as this permitted assessment of the relative performance of laboratory-reared and field mosquitoes under the same thermal regime. It is possible that had we chosen to artificially manipulate temperature and humidity conditions within the known acceptable range, we might have found some combinations in which the apparent fitness deficit between laboratory and field mosquitoes could have been reduced or reversed. The task for those involved in field release trials of laboratory insects is to identify if and what these conditions may be. What is clear from the present study is that rearing conditions typical of most laboratory colonies do not generate mosquitoes that are better provisioned than those in the wild.

A final credo to these conclusions is that they have been reached by considering only physiological determinants of survival. Equally as important may be behavioral or genetic factors that alter the relative performance of these phenotypes in nature, independently of the base differences in energetic provisioning reported here. Previous control efforts based on releasing laboratory-reared males suggest these behavioral factors would give an advantage to field males (Benedict and Robinson, 2003; Ferguson et al., 2005), a similar conclusion to what we predict from physiology. Clearly current insect rearing protocols need to be improved to enhance the quality of males produced, to the point where they at least match, if not exceed, the body size and energetic make-up of wild individuals. Further studies to explore the intrinsic determinants of the mating success and survival determinants in wild insects, especially those that are the target of genetic control for disease control, are strongly encouraged.

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